Chapter 3
Developmental Biology and Regeneration

A1a. Identify and characterize hepatic stem cells in fetal and adult liver. The presence of hepatic progenitor cells has been identified in adult liver by detection of Hedgehog signaling which is active during embryogenesis of the liver, but not found in fully differentiated hepatocytes and cholangiocytes (Sicklick JK, *Am J Physiol Gatrointest Liver Physiol,* 2005: In press). Two types of endodermal progenitor cells contribute to the liver bud in embryos (Tremblay KD. *Dev Biol.* 2005;280:87). (20%)

A1b. Profile transcriptional network during endodermal specification, liver growth and regeneration. Microarray analyses of different stages of liver development and regeneration are being evaluated by NIH-funded investigator-initiated research project grants, but there has yet to be a synthesis of results. (0%)

A2a. Identify noninvasive biomarker or imaging method for assessing liver regeneration. Whole liver imaging for liver volume is still used as a means of assessing human liver regeneration after hepatectomy and partial liver graft transplantation. Development of markers for regeneration is encouraged in the following program announcements: "Non-Invasive Methods for Diagnosis and Progression" (PA-04-088) and "Development of Disease Biomarkers" (PA-05-098). (0%)

A2b. Define role of inflammation, fibrosis, and cell injury in regeneration. Many pro-inflammatory signals play a role in liver regeneration, including IL6, TGFβ, and lymphotoxin (Anders RA. *J Immunol* 2005;175:1295). The interplay of these signaling pathways and their modulation as well as the role of fibrogenic pathways in regeneration require further elucidation. (10%)

A3a. Define role of nonparenchymal cells in liver regeneration and liver development. The extracellular matrix, a major product of nonparenchymal cells, plays an essential role in normal liver regeneration (Serandour AL. *Hepatology* 2005;41:478). (10%)

A3b. Develop new animal model systems to study liver development. Both the frog (Xenopus) and zebrafish are being used to assess liver embryogenesis and have already delineated roles for several pathways in liver development, including FGF and secreted frizzled-related protein 5, HNF1β, 4 and 6, and the Wnt and Notch signaling pathways (Lemaigre F. *Curr Op Genet Dev* 2005;14:582). (20%)

B1a. Develop methods to select transplanted donor cells and induce homing and engraftment of transplanted cells to the liver. Gene therapy of liver disease would be benefited by the development of means to target cells to the liver. (0%)

B1b. Identify how deregulation of genes and pathways involved in normal regeneration contributes to carcinogenesis. The forkhead transcription factor Foxm1b is induced during hepatocyte proliferation and is necessary for liver

morphogenesis. Overexpression of Foxm1b promotes liver cancer in mice; therefore, modulation of Foxm1b expression may be an approach to prevention or treatment of hepatocellular carcinoma (Costa RH. *Curr Op Genet Dev* 2005;15:42. (10%).

B2a. Validate biomarkers of regeneration in living donor liver donation and acute liver failure. This area of research is encouraged directly in a program announcement on "Development of Disease Biomarkers" (PA-05-098) and is the focus of ancillary studies in the A2ALL cohort study of living donor liver transplantation. (0%)

B2b. Identify pathways that stop proliferation of hepatocytes as liver returns to normal mass. Factors that control or stop hepatocyte proliferation in animal and cell culture models include TGFβ, p53, and the cyclin regulatory genes (Romero-Gallo J. *Oncogene* 2005;24:3028). The role of these factors in human liver regeneration awaits study. (10%)

B3. Delineate sequence of molecular and cellular events that lead embryonic stem cells to differentiate into mature hepatocytes. Delineation of the molecular events that lead to differentiation of stem cells to mature hepatocytes is the focus of much basic research in animal models and the Foxm1b transcription factor appears to be a major component. Delineation of steps in this transition in human hepatocytes has not yet been accomplished. (10%)

C1. Develop *ex vivo* and *in vivo* vectors for liver-directed gene therapy. Research on vectors for gene therapy is encouraged through the NIH Molecular Therapy Centers programs. (0%)

C2a. Develop safe means of promoting normal liver regeneration for acute liver failure, liver resection, and transplantation. Progress in this area will require the initial delineation of the cellular pathways that lead to normal liver regeneration and application of cytokines or small molecules that promote normal regeneration. Candidates include IL-6, TNFα, HGF, EGF, and TGFα. Several of these molecules have been used in animal models of regeneration but none have been applied to humans. (0%)

C2b. Delineate molecular and cellular events that lead from endodermal liver primordium to mature liver in fetal development. The movement of the hepatic endoderm towards the site of liver bud formation and the position of the endoderm as it is exposed to different mesodermal signals for liver development have been mapped (Tremblay KD. *Dev Biol* 2005;280:87). However, the molecular signals accompanying each step require further elucidation. The endodermal transcription factors GATA-6, FOXA1, and FOXA2 are crucial for early liver development (Zhao R. *Mol Cell Biol* 2005;25:2622; Lee CS. *Nature* 2005;435:944). (30%)

C3a. Develop practical gene or cell therapy for metabolic liver disease. Pilot studies of innovative gene and cell therapy for several metabolic liver diseases have been conducted, but practical approaches have yet to be developed. (0%)

C3b. Develop *in vitro* model of hepatic organogenesis. Three dimensional systems for growing hepatocytes have been developed that may ultimately be applicable to use in a hepatic assist device (Monga SP. *Am J Pathol* 2005;167:1279). (10%)

Figure 5. Estimated Progress on Developmental Biology and Regeneration Research Goals, 2005 (Year 1)